molecular biology

Restorase™: A Novel DNA Polymerase Blend That Repairs Damaged DNA

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Application Notes

- Repairs damaged DNA
- Amplifies sequence where other thermostable polymerases fail
- Amplifies sequences in multiplex reactions
- Increases amplicon specificity and yield
- Applicable over a wide range of amplicon size (200 bp to 20 kb)

Introduction

DNA template quality is a critical factor in the successful amplification of target sequences via PCR. DNA is often damaged when stored or exposed to substandard conditions such as acid, alkylating agents, heat, light, phenol/chloroform extraction, reactive oxygen species or simply time. Standard storage and extraction procedures result in base damage and/or loss that can impede primer annealing, polymerase fidelity and polymerase processivity, resulting in inefficient or failed amplification.

This discussion highlights both the increased yield and specificity of PCR amplification from either damaged or undamaged DNA template using Restorase, Sigma's new DNA repair and amplification polymerase blend. Restorase combines a DNA repair enzyme with Sigma's high-quality long PCR DNA polymerase blend to repair damaged DNA sites. The result is an increased amplicon specificity and yield. Restorase is designed to amplify long DNA fragments from damaged template DNA that is unable to be amplified using standard PCR enzymes. Restorase improves both the yield and specificity of PCR amplification from both damaged and undamaged template and has been proven effective on amplicons ranging from 200 to 20,000 bp.

Repair and amplification of highly degraded DNA template

Apurinic/apyrimidinic (AP) sites represent some of the most common forms of DNA damage. To recreate this damage, Lambda (λ) DNA was treated with formic acid for 7.5 minutes and 10 minutes (Figure 1). The ability of Restorase to amplify a 742 bp fragment of DNA from these samples was compared to that of *Taq* DNA polymerase. Restorase increased amplicon yield over *Taq* when amplifying from the 7.5-minute formic acid treated DNA, and produced ample product from the 10-minute formic acid treated DNA that *Taq* was unable to amplify.

Rescue of correct mouse genotyping

Meaningful results often rely on the accurate genotyping of experimental organisms during the early stages of an experiment. Incorrect genotyping can result in the loss of both time and rare experimental samples. In Figure 2, we tested the ability of both *Taq* DNA polymerase and Restorase to correctly genotype both Substance P (Sub. P) positive and negative (knockout) mice from phenol chloroform extracted mouse genomic DNA samples. A 289 bp fragment of fatty acid binding protein (FABP) was amplified (positive control) in multiplex reactions with a 627 bp fragment of Sub. P. Pups 1 and 24 are wild type (contain sequences for both FABP and Sub. P), while pup 2 is a Sub. P knockout (contains only FABP sequence). While Restorase correctly identified both wild type mice, *Taq* DNA polymerase failed to amplify detectable Sub. P bands, potentially leading to the incorrect genotyping of both wild type mice.

Enhanced yield and specificity

Specificity and yield are two important considerations of PCR performance. The inefficient amplification of sequence by an inferior polymerase can lead researchers to increase PCR cycle number, thus increasing the probability of misincorporation of nucleotides. This can be problematic when ultimately sequencing a PCR product. Likewise poor specificity can result in numerous unwanted bands, both interfering with and confusing the interpretation of experimental results. To compare yield and specificity of AccuTaq™ and Restorase, a 5 kb fragment of human genomic DNA was amplified from 0, 1, 3, 5, 7, and 10 minute formic acid treated samples. Restorase produced ≥ 2-fold increase in amplicon yield (Figure 3, quantification data not shown) and greatly increased primer specificity in all samples tested.

Summary

Restorase allows for the amplification of highly degraded DNA samples unable to be amplified using other polymerases. As it increases both yield and specificity in all samples tested, it is also a powerful enzyme blend for day-to-day use. As demonstrated by experimental results, Sigma's Restorase is a powerful and superior enzyme blend in the amplification of both damaged and undamaged DNA.

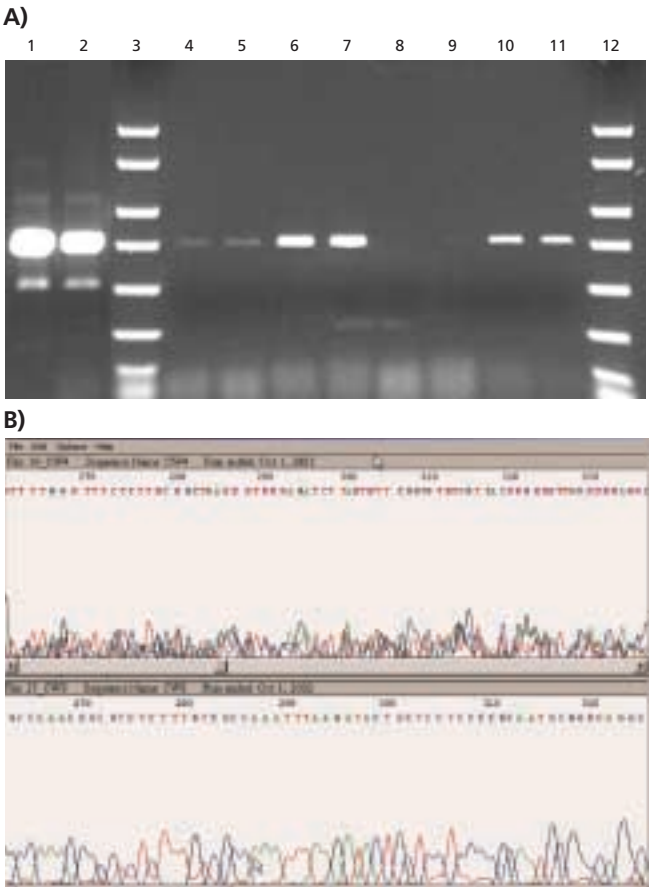

Figure 1. Restorase allows for PCR amplification of DNA that could not be amplified using* Taq *DNA polymerase. *Lambda (λ) DNA was damaged with formic acid for 7.5 minutes and 10 minutes.* ***A)*** *A 742 bp DNA fragment was amplified from undamaged DNA (Lanes 1 + 2), 7.5-minute damaged DNA (Lanes 4-7), and 10-minute damaged DNA.* Taq*-amplified products were run in duplicate in Lanes 1-2, 4-5, and 8-9, while Restorase-amplified products were run in duplicate in Lanes 6-7 and 10-11. Lanes 3 and 12 show PCR markers (2000, 1500, 1000, 750, 500, 300, 150, and 50 bp).* ***B)*** *Sequencing of* Taq*-amplified product from 7.5-minute damaged template (Lane 3) resulted in a high percentage of miscalls and unreadable sequence (top picture), while sequencing of Restorase-amplified product from 7.5-minute damaged template (Lane 5) resulted in high-quality sequence (bottom picture).*

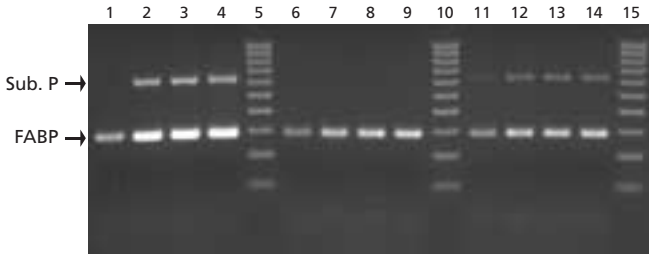

Figure 2. Restorase rescues correct genotyping where regular* Taq *DNA polymerase fails. *Lanes 5, 10, and 15 show 100 bp PCR ladder. Lanes 1-4 show products amplified from wild type pup #1, Lanes 6-9 show products amplified from Sub. P knockout pup #2, and Lanes 11-14 show products amplified from wild type pup #24. Products in Lanes 1, 6, and 11 were amplified using* Taq*, failing to produce Sub. P bands resulting in the incorrect genotyping of pups #1 and 24. Products in Lanes 2-4, 7-9, and 12-14 were amplified using Restorase, producing proper bands in all cases resulting in the proper genotyping of each mouse. Restorase samples for each mouse were pre-incubated with Restorase PCR mix at 0 °C for increasing amounts of time (from left to right: 1, 3, and 5 minutes, respectively).*

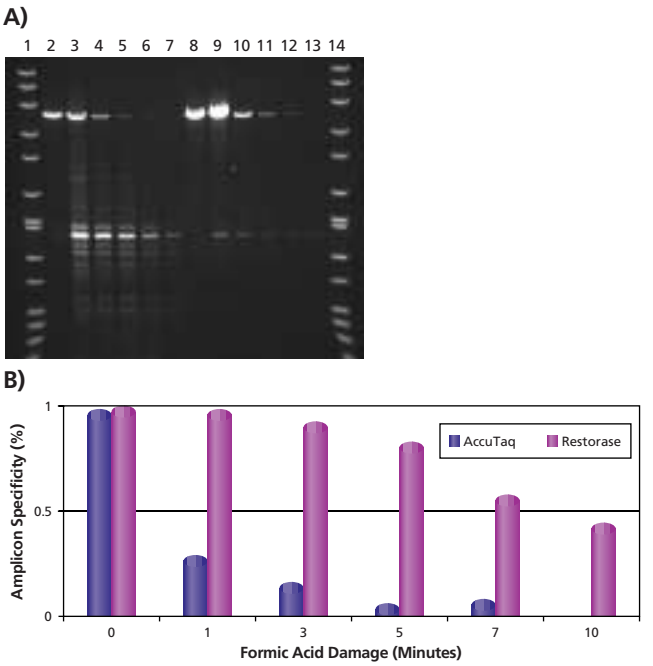

Figure 3. Restorase increases both yield and specificity over AccuTaq. A) *Human genomic DNA was damaged with formic acid for 1, 3, 5, 7, and 10 minutes. Lanes 1 and 14 show PCR markers (10, 8, 6, 4, 3, 2, 1.5, 1.4, 1, 0.75, 0.5, 0.4, 0.3 kbs). Primers used were designed to target a 5 kb fragment of human genomic DNA. Products in Lanes 2-7 were amplified using AccuTaq, while products in Lanes 8-13 were amplified using Restorase. Lanes 2 and 8 show product amplified from undamaged template, Lanes 3 and 9 show product amplified from 1-minute damaged template, Lanes 4 and 10 show product amplified from 3-minute damaged template, Lanes 5 and 11 show product amplified from 5-minute damaged template, Lanes 6 and 12 show product amplified from 7-minute damaged template, and Lanes 7 and 13 show products amplified from 10-minute damaged template.* ***B)*** *Restorase increases amplicon specificity compared to AccuTaq. Amplicon specificity was measured as a percentage of specific product (5 kb amplicon yield / sum of all non-specific amplicon yields), and was obtained from the reactions performed in Figure 3A above.*

Ordering Information

Product	Description	Unit
R 1028	Restorase DNA Polymerase with 10x reaction buffer	50 reactions 200 reactions